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The Effects of Growth Regulators on Development of *Nicotiana affinis* Flowers *In Vitro*

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Additional index words. kinetin, NAA, IAA, IBA, flowering tobacco

Abstract. **Excised flower buds of *Nicotiana affinis* were grown to maturity in media in which growth regulators were added to evaluate their effects on growth and development. As the concentration of naphthaleneacetic acid (NAA) was increased from 10^{-7} to 10^{-4} M, the number of days required for the buds to open decreased, and the number of days required for the corolla tubes to turn brown increased. Indoleacetic acid (IAA) and indolebutyric acid (IBA) did not affect the rate of bud opening. As the concentration of IBA was increased, however, the days required for the corolla tubes to turn brown increased, but not as much as for buds in media with NAA. IAA had no effect on browning of corolla tubes. At high concentrations of NAA and kinetin the corolla tubes were shortened. Kinetin did not affect the rate of bud opening or days till the corolla tubes turned brown.**

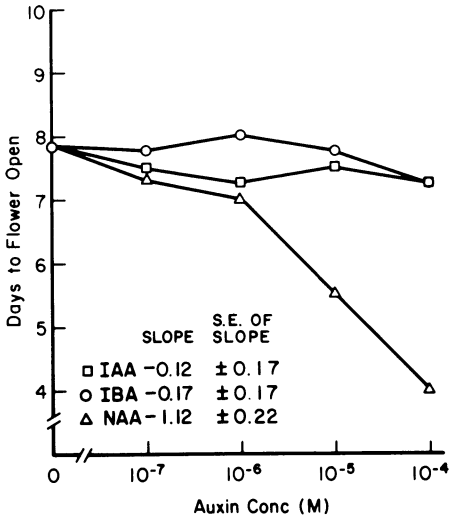

Fig. 1 Effect of concentration of auxin on number of days for buds to open after being placed into flasks.

Excised buds of several species of plants have been cultured successfully to fully opened flowers *in vitro* (2, 3, 4, 5, 10, 11). *In vitro* culture of flower buds permits the study of the effects of growth regulators and other substances on flower development and senescence under sterile controlled conditions without possible interfering effects of substances produced in stems, leaves, and roots.

Exogenous auxin and cytokinin have been shown to affect floral morphogenesis *in vitro*. IAA promoted normal petal development on excised buds of *Aquileqia* (10). In a later study (3), however, IAA was either ineffective in modifying *Aquileqia* floral development or inhibitory at the highest concentration used. NAA and 6-benzylaminopurine (BA) (7) initially were found to be required for flower formation in *Passiflora*, but later studies (8) showed that only BA was necessary. Kinetin was not required for floral bud initiation of tobacco but was required for advanced stages of bud growth and development in another study (5). In the present study, the effects of auxin and kinetin on flower development and senescence of *Nicotiana affinis* (Moore) were evaluated *in vitro*.

Nicotiana affinis 'Nicki Red' plants were greenhouse grown with 18°C-night and 24° to 26°-day temperatures. Buds, about 2 cm in length, were harvested from the plants for this study. Preliminary studies were conducted to determine the size of bud to use in these experiments. Buds 2 cm in length had visible, but undeveloped, pistils and a stamen. Buds much smaller than 2 cm would not open normally.

Liquid (2, 10) Murashige-Skoog medium (6) supplemented with 5% sucrose and adjusted to pH 5.8 was added to 250 ml Erlenmeyer flasks. Flasks were plugged with cotton and autoclaved for 20 min at 1 kg/cm^2 and 120°C. Buds were surface sterilized in a 0.5% sodium hypochlorite solution for 3 min, rinsed twice in sterile water, and then transferred to sterile flasks. Each flask contained a bud support constructed of nichrome wire. The cultures were maintained at 26° under continuous fluorescent light at about 100 μmol $s^{-1}m^{-2}$ (LI-COR 185 light meter and LI-190S quantum sensor) for the duration of the experiment.

Expt. 1 tested the effects of kinetin and NAA on flower development and senescence. A stock solution containing 10^{-3} M kinetin in 0.5% dimethylsulfoxide (DMSO) was prepared (9). Final kinetin concentrations in 30 ml of test solutions were 10^{-4}, 10^{-5}, and 10^{-6} M. A stock solution containing 10^{-3} M NAA was prepared by dissolving NAA in 20 drops of 1N NaOH and then

Table 1. Effect of concentration of NAA and kinetin on number of days for buds to open after being placed in flasks.

	Days to flower				
	NAA Concn (M)				
Kinetin concn (M)	0	10^{-7}	10^{-6}	10^{-5}	10^{-4}
0	7.7	6.8	7.0	4.8	4.2*
10^{-6}	8.8*	7.5	6.8	5.2	5.2
10^{-5}	8.2*	7.5	6.8	5.0*	4.7
10^{-4}	7.8	8.0	5.7	5.8	5.3

*Means which are an average of 5 replications; all other means have 6 replications.
SE for means with 5 replications = ± 0.6. SE for means with 6 replications = ± 0.5.

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Table 2. Effect of concentration of NAA and kinetin on corolla tube length.

	Length of corolla tube (cm)				
	NAA concn (M)				
Kinetin concn (M)	0	10^{-7}	10^{-6}	10^{-5}	10^{-4}
0	3.00	2.95	2.88	2.45	2.42
10^{-6}	3.12	2.90	2.77	2.73	2.18
10^{-5}	2.90	2.62	2.72*	2.22	2.33
10^{-4}	1.90	1.95	1.75	1.78*	1.75

*Means which are an average of 5 replications; all other means have 6 replications.
SE for means with 5 replications = ± 0.13. SE for means with 6 replications = ± 0.12.

diluting to final volume. Final concentrations of NAA were 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} M in 30 ml of medium. Growth regulators were added to the medium by filter sterilization to reduce the potential for breakdown of the growth regulators due to autoclaving. A factorial combination of kinetin and NAA was used to test the interactive effects. The experiment was conducted twice using a randomized complete block design with 3 replications.

Expt. 2 was conducted to compare effects of IAA, IBA and NAA on flower development and senescence. The final concentrations of the auxins in the medium were 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} M in 30 ml of medium. The solutions were prepared as in expt. 1. The split-plot experimental design, with auxins as the main plot and auxin concentration as the subplot, was replicated 4 times.

Data were collected for the following characteristics: Days to open—number of days after initiation of cultures until the petals were extended perpendicular to the corolla tube; Elongation—length of the corolla tube at the time of flower senescence; and Days to brown—number of days after initiation of cultures until corolla tube turned brown.

The analysis of variance for the 2 experiments tested for linear and curvilinear relations between an increase in growth regulator concentration and data collected.

Expt. 1: Kinetin did not alter significantly the days required for the buds to open, and no interaction was observed between kinetin and auxin. Therefore, the data for kinetin were pooled to evaluate the effect of NAA on bud opening (Table 1). Increasing the NAA concentration from 10^{-7} M to 10^{-4} M resulted in a significant (5% level) linear decrease in the number of days for the buds to open. Buds cultured in NAA at any concentration opened significantly earlier than the control. At the highest concentration of NAA (10^{-4} M), the buds opened in an average of 4.8 days; whereas buds in media without NAA required 8.1 days to open.

The length of the corolla tubes was affected by kinetin and NAA (Table 2). Regardless of the level of NAA, there was significant (5% level) decrease in the length of the corolla tubes when the kinetin concentration increased from 10^{-5} M to 10^{-4} M. The difference in corolla tube lengths between buds cultured in 10^{-5} M and 10^{-4} M ranged from about 0.5 to 1 cm depending upon the NAA concentration. The corolla tubes of buds cultured in media with 10^{-5} M or 10^{-6} M kinetin were not different from those of the control. As the concentration of NAA was increased from 10^{-7} M to 10^{-4} M, there was a significant (5% level) linear decrease in the length of the corolla tubes. Buds cultured in media containing 10^{-4} M NAA averaged 0.5 cm shorter than buds in media without NAA.

Kinetin also had no affect on the number of days required for the corolla tubes to turn brown (Table 3). Therefore, the data for kinetin were pooled to evaluate the data for NAA treatments. As the NAA concentration was increased from 10^{-7} M to 10^{-4} M there was a significant (5% level) linear increase in the number of days required for the corolla tubes to turn brown. Buds in media containing 10^{-7} M NAA required the same number of days to turn brown as the control; however, if the NAA concentration was increased to 10^{-4} M the corolla tube turned brown about 9 days after the buds without NAA.

Expt. 2: Since NAA exhibited a marked effect on flower maturation and on delaying senescence in Expt. 1, IAA and IBA were compared to NAA in a 2nd experiment. Kinetin was not included in Expt. 2 because it had little effect on flower bud development in Expt. 1. As in Expt. 1, there was a significant linear (5% level) decrease in number of days to flower opening as the concentration of NAA was increased (Fig. 1). Buds cultured with IAA or IBA were not significantly different from buds cultured in media with no auxin. Buds without auxin in the medium opened after 7.8 days in culture; at 10^{-4} M NAA, the buds were open after only 4.1 days.

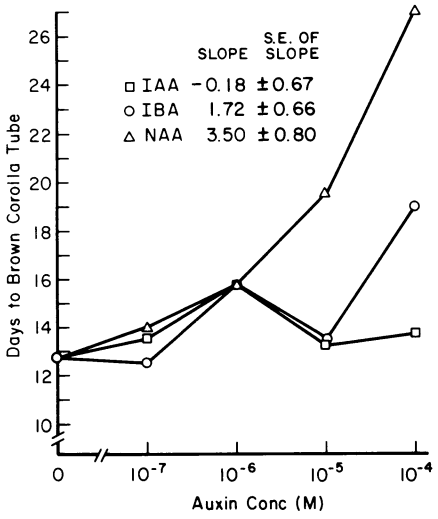

Fig. 2 Effect of concentration of auxin on the number of days after flowers were placed into flasks until the corollas turned brown.

As the concentration of NAA and IBA was increased from 10^{-7} to 10^{-4} M there was a significant linear (5% level) increase in the number of days to browning of the corolla tubes (Fig. 2). Although not significant at the 5% level, there is an indication that the slope of the NAA concentration curve is greater than the slope of the IBA concentration curve. The slope of the IAA concentration curve was not significantly different from that of the control.

The effects of NAA in these experiments on flower opening and elongation differ from several previous reports in which auxins were reported to have no effect on growth of buds (2, 4). In each of the previous studies the buds, when placed in culture, were probably less mature than buds used in this study. In addition, the endogenous auxin level may be sufficient for bud development in some species but not in others.

The high concentration of kinetin used in the present study caused the petals of flowers to become partially fused. Ballantyne (1) noted similar abnormal development with high kinetin concentrations in experiments with *Narcissus*. Because kinetin's only significant effect on flower development in this study was to shorten the corolla tube, it seems that kinetin was not required for growth of *Nicotiana affinis* flowers in culture. Blake (4) also found no effect of cytokinins on flower growth of *Viscaria* cultured from buds. Cytokinins have been reported to be necessary for normal flower bud growth in culture in other plants (2, 3, 11). As Berghoef and Bruinsma (2) point out, different results might be explained by the development stage of the buds used in different studies. If the buds are

Table 3. Effect of concentration of NAA and kinetin on number of days after flowers were placed into flasks until the corolla tubes turned brown.

	Days to brown corolla tube				
	NAA concn (M)				
Kinetin concn (M)	0	10^{-7}	10^{-6}	10^{-5}	10^{-4}
0	12.8	13.5	15.7	18.2	23.8*
10^{-6}	13.2	14.5	18.0	20.3	20.7
10^{-5}	14.0	13.0	14.3	22.0	22.2
10^{-4}	13.8	11.6*	13.3	16.7	24.0

*Means which are an average of 5 replications; all other means have 6 replications.
SE for means with 5 replications = ± 2.1. SE for means with 6 replications = ± 1.9.

relatively mature when cultured, most cell divisions necessary for flower development will have occurred. The buds used in this study were possibly more mature than in other studies. Even if all cell divisions have not occurred, endogenous cytokinin content may be sufficient to sustain the remaining development.

Nicotiana affinis flowers can be successfully cultured *in vitro*. This botanically simple flower may be useful as a model for *in vitro* studies of flower development and senescence.

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In Vitro Propagation of Prayer Plants

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Additional index words. micropropagation, shoot tip culture, *Maranta leuconeura*

Abstract. **Lateral buds excised from unrooted cuttings of *Maranta leuconeura* E. Moor. 'Kerchoviana', prayer plant, were cultured on Linsmaier and Skoog (LS) medium supplemented with various combinations of N6-benzylaminopurine (BA), kinetin, α-naphthaleneacetic acid (NAA), and 3-indoleacetic acid (IAA) for the production of multiple shoots and complete plants. Of 48 combinations of growth regulators, 4 produced the most vigorous, well-formed shoots after a culture period of 6 weeks: 2.0 mg·liter^{-1} kinetin, 0.2 mg·liter^{-1} BA, 0.2 mg·liter^{-1} BA plus 0.1 mg·liter^{-1} NAA, and 2.0 mg·liter^{-1} kinetin plus 1.0 mg·liter^{-1} IAA. Enhanced shoot production occurred when shoots were transferred at 12-week intervals and when they were maintained on 0.2 mg liter^{-1} BA. Complete plants with strong, fibrous root systems were produced after the shoots were transferred to basal medium lacking growth regulators for 3–4 weeks.**

Maranta leuconeura 'Kerchoviana', prayer plant, is normally propagated asexually by stem cuttings because seeds rarely germinate (1). Cuttings are rooted in humidity tents for 4–6 weeks depending on the time of year. It is estimated that 15% to 20% of the cuttings produce inferior root systems and must be discarded, since developing roots are unable to penetrate leaf sheaths uniformly, and lateral root growth is inadequate. Conventional cuttings are unacceptable for use in terrariums or as small potted plants because they develop large leaves and elongated stems. (Tom Harcharik, Plant Manager, Yoder Bros., Salinas, Calif., personal communication). Since prayer plants are a commercially desirable foliage plant, it would be advantageous to produce large numbers of compact prayer plants, without losses, for use in terrariums and in response to market demands for plants which can be grown in small spaces. The objective of this study was to develop *in vitro* techniques (2) which produce attractive, compact plants with strong, fibrous root systems suitable for commercial production.

Aseptic culture. Prayer plant cuttings were obtained from a commercial source. Rooted and unrooted cuttings (20–25 cm long) were used as explant sources. After leaf blades and roots were removed, stems were soaked in 1.05% NaOCl solution (prepared as a 20% solution of commercial bleach) plus 0.02% Triton X-100 for 15 min with intermittent shaking. A 2nd application of dilute bleach (1.05% NaOCl) was applied for 5 min. Stems were transferred into sterilized jars and rinsed 3 times with sterile deionized water. Lateral buds (2–5 mm long) were excised under aseptic conditions and were placed on LS basal medium (3). The pH of the medium was adjusted to 5.8, and the medium was solidified with 0.8% Difco Bacto agar and autoclaved for 15 min at 121°C and 1.1 kg·cm^{-2}. The excised buds and subcultured shoots were placed in 25 × 150 mm culture tubes containing 15 ml LS medium. All cultures were incubated in controlled environment chambers at 26° ± 2°C under cool-white fluorescent lights providing 78 ± 6 μmol s^{-1}m^{-2} under a 16-hr photoperiod.

Effects of growth regulators on shoot development. Combinations of exogenously applied auxins (NAA and IAA) and cytokinins (kinetin and BA) were compared for their effects on shoot and root development (4). Kinetin at 1, 2, 3, and 5 mg·liter^{-1} and BA at 0.1, 0.2, 0.5, and 1.0 mg·liter^{-1} were each combined with NAA at 0, 0.1, and 1.0 mg·liter^{-1} and IAA at 0, 1.0, and 2.0 mg·liter^{-1} in an 8 × 6 factorial design to make a total of 48 treatments. Each of the treatments was replicated 5 times on explants from both rooted and unrooted cuttings. Since contamination of explants from rooted cuttings was 89% compared to 22% from unrooted cuttings, only data on explants from unrooted cuttings were analyzed. Data were subjected to an analysis of variance ($P = 0.05$).

Four growth regulator treatments, 2.0 mg·liter^{-1} kinetin, 0.2 mg·liter^{-1} BA, 0.2 mg·liter^{-1} BA plus 0.1 mg·liter^{-1} NAA, and 2.0 mg·liter^{-1} kinetin plus 1.0 mg·liter^{-1} IAA, produced the most vigorous and well-formed shoots. After 6 weeks in culture, large variations in plant morphology, root and shoot proliferation, and overall vigor were observed.

One hundred random lateral shoots (10–15 mm long) were subcultured on each of the 4 growth regulator treatments, 2.0 mg·liter^{-1} kinetin, 2.0 mg·liter^{-1} kinetin plus 1.0 mg·liter^{-1} IAA, 0.2 mg·liter^{-1} BA, and 0.2 mg·liter^{-1} BA plus 0.1 mg·liter^{-1} NAA, in order to determine which was the most appropriate growth regulator combi-

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